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THE FINER HISTOLOGY OF THE NORMAL GLOMERULUS

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LEONE McGREGOR

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THE FINER HISTOLOGY OF THE NORMAL GLOMERULUS*

LEONE MCGREGOR, PH.D., M.D.

(From the Department of Pathology, University of Minnesota, Minneapolis, Minn.)

INTRODUCTION

The object of this paper is to review the literature and to present some new facts regarding the histology of the normal human renal glomerulus.

I. REVIEW OF LITERATURE

Bowman's Capsule: In 1688 Malpighi discovered the renal glomerulus by means of injections through the renal artery. He considered it a gland which might possibly give origin to the uriniferous tubule. Schumlansky, 1782, suggested in a diagram some connection between glomeruli and tubules. Littre, 1705, and Johannes Müller, 1830, were the first to describe the capsule. Huschke, 1828, and Müller believed the capsule to be closed except where the vessels entered. Bowman, 1842, proved the union of tubule and glomerulus by a method of successive injection of saturated solutions of potassium bichromate and lead acetate. In both human and animal kidneys the precipitate which formed in the vessels often burst through the glomerular tuft and passed down the tubule. He described the capsule as consisting of an external tunic of transparent homogeneous tissue lined by epithelium. These epithelial cells were non-ciliated, flat and continuous with those of the convoluted tubules. These findings were confirmed in 1845 by Bidder who also noted the continuity of the capsular and tubular basement mem-

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branes. Roth, 1864, and His, 1865, were the first to demonstrate cell boundaries with silver preparations. Ludwig, 1872, described the capsule as consisting of "a mosaic of cells closely resembling those composing the wall of the blood and lymphatic capillaries with a little connective tissue around the outer surface." Drasch, 1877, saw that the nuclei of adjacent cells lay side by side in eccentric positions. Herring, 1900, after a comprehensive review of the embryology of the kidney, concluded that the cavity of Bowman's capsule should be regarded as a body cavity, especially differentiated for urinary secretion.

Mall, 1896, showed the capsular basement membrane to be composed of longitudinally and circularly disposed reticular fibrils, which when very dense and compressed, gave a homogeneous appearance. They anastomosed with the fibrils of adjacent tubules. In spite of these facts, von Ebner in 1902 described the membrane as structureless and glassy. In 1902 Mall was able to show that there were two basal membranes, an inner one homogeneous and structureless, applied to the bases of the epithelial cells, and an outer one interlacing and fibrillar. The latter was part of the reticular stroma of the kidney. Rühle, 1897, Russakoff, 1908, and Corner, 1920, confirmed Mall on these points. Corner believed the fibrillar membrana propria to be produced by the endothelium of adjacent capillaries. von Frisch, 1915, although agreeing that the tubules have a double basement membrane, definitely stated that the capsular membrana propria is a structureless glassy skin. Schäffer, 1927, concluded that the membrane must be considered as a cuticular excretion of the epithelial cells.

Glomerular Endothelium: The history of the cytology of the glomerular tuft begins with Bowman, 1842. He described the vascular loops as lying naked within the lumen of the capsule, held together only by their mutual interlacement. "The Malpighian capillary system stands alone among similar structures in being bare." Among his supporters were Johnson, 1850, Frerichs, 1851, and Henle, 1873. Henle stated: "The vessels in the glomerulus have the calibre of capillaries. Their framework is a structureless wall possessing elliptical nuclei." Many attempts were made to find cell boundaries between these capillary nuclei. Chrzonszczewsky, 1864, Ludwig, 1872, Drasch, 1877, Ribbert, 1879, Hortoles, 1881, and Buday, 1906, after numerous silver injections, were unsuccessful although they

could easily demonstrate boundaries between the endothelial cells of the vas afferens and vas efferens. Langhans, 1879, described the endothelial layer as having very few nuclei. Eliaschoff, 1883, found that these nuclei were more conspicuous in rabbits than in other animals. In 1886, Nussbaum demonstrated cell boundaries in the endothelium of the frog's glomeruli. These cells projected into the lumen and were very distinct in cross-section. von Moellendorff, 1927, described the endothelium of the human glomerulus as a fine layer with a few nuclei and visible cytoplasm only where such nuclei lay. He could not find cell boundaries.

Glomerular Epithelium: The epithelial covering of the glomerular tuft was first described by Gerlach in 1845 and 1848. By injections through the ureter he was able to show that the vascular loops were separated from the capsular space by cells which he believed were the essential elements of a gland. These cells formed a layer over the tips and crevices of the glomerulus. Bidder, 1845, suggested but could not prove conclusively the presence of glomerular epithelium. Carus, 1850, von Wittich, 1851, Koelliker, 1854, McDonnell, 1855, Chrzonszczewsky, 1864, Duncan, 1867, and Toldt, 1874, all supported Gerlach. In 1857 Isaacs injected glomeruli by way of the ureter and tubules until the capsules ruptured. With a dissecting microscope he examined through the hole in the capsule the epithelial covering of the loops. His illustrations showed how much larger these cells were than those lining Bowman's capsule. Moleschott, 1862, measured the glomerular and capsular epithelial cells and found the diameters to average 0.01 and 0.006 mm., respectively. Steudener, 1864, saw a fine covering over the glomerular loops but could not recognize it as epithelium. Schweigger-Seidel, 1865, and Seng, 1871, observed the presence of epithelium over the tips of the loops in the embryo. Ludwig, 1872, saw that the walls of the capillaries were separated from the capsular contents by "a layer of not very well defined cells with spherical nuclei." He was not sure that these were continuous from lobule to lobule. R. Heidenhain, 1874, injected the renal arteries of cats, birds, rats, field mice, rabbits, guinea pigs, sheep, pigs and cows, and found the epithelial cells forming a continuous layer over and between the loops. His work was confirmed by the findings of Frey, 1875, Langhans, 1879, Ribbert, 1879, and Nauwerck, 1884. Ribbert stated that the numerous nuclei observed in a normal glomerulus were for the most

part epithelial. Drasch, 1877, and von Ebner, 1902, believed this epithelium was in the form of a syncytium. Ziegler, 1879, preferred to consider it as endothelium comparable to the lining of the serous cavities. Nussbaum, 1886, clearly distinguished the large glomerular epithelium from the smaller endothelium. He believed the epithelial cells were often multinucleated and that amitosis occurred in them. He recognized the continuity of glomerular and capsular epithelium. Hansemann, 1887, with Isaac's technique isolated the glomerular epithelium and described the cells as flat, often multinucleated, and taller than those lining the capsule. He pointed out the more rapid postmortem digestion of the epithelial than of the endothelial cells. In 1900, Herring's review of the development of the glomerulus explained completely the position and derivation of glomerular epithelium. In a 1909 Harvey lecture, Huber stated that the columnar glomerular epithelium of the embryo became pavement epithelium in the adult. Mertz, 1918, in a quantitative study of the cells in the average 10 micron section of a human glomerulus, was unable to find sufficient morphological difference between endothelium and epithelium to count them separately. Löhlein, 1910, Gross, 1919, Fahr, 1925, Munk, 1925, and Elwyn, 1926, all described glomerular epithelium and its pathological changes. Gross preferred to think of it as a syncytium rather than true epithelium. Fahr stated that glomerular epithelium was so closely related to tubular that it would seem to be secretory. Zimmerman, 1911, 1915 and 1923, demonstrated boundaries between these cells in cats and hedgehogs, but preferred to call them pericytes. von Moellendorff, 1927, described the "deck" cells or glomerular epithelium as morphologically similar to the adventitial cells or pericytes of the capillary bed. He believed these cells have long, branching, anastomosing processes which form a network over the glomerular loops. He ascribed to their mesenchymal origin, their ability to take the form of adventitial cells and to transform into free round cells in pathological conditions. Vimtrup, 1928, adhered to the older conception of an epithelial covering closely applied to the surface of each capillary loop. Volterra, 1928, described these cells as perithelial but did not see any processes on them. He concluded that the cell branches described by von Moellendorff belonged to the glomerular basement membrane and were a part of the connective tissue adventitia of the capillary tuft. Bargmann, 1929, studied the glomeruli of many species and believed

with von Moellendorff that the branching processes belonged to the "deck" cells.

Glomerular Basement Membrane: The possibility of a membrana propria in the glomerular loops was evidently considered by Bowman and rejected for he wrote: "The capsular basement membrane is perforated by the afferent and efferent vessels and is certainly not reflected over them." Seng, 1871, saw and illustrated the presence and continuity of the glomerular and capsular basal membranes. Toldt, 1874, confirmed this finding in both embryonic and adult human glomeruli. Drasch, 1877, after injection of silver nitrate into the glomerular vessels, saw a formation which depended on a porous condition in a membrane. Langhans' illustrations, 1879, show a basement membrane which he described as a very fine doubly-contoured border. Nussbaum, 1886, after studying the embryology of the malpighian body, searched unsuccessfully for a glomerular membrane and concluded that the wall of a glomerulus had only two layers, endothelial and epithelial. Mall, 1896, after boiling kidney tissue in water, digested frozen sections with pancreatin and found a homogeneous striped membrane still present in the glomerular loops. Rühle, 1897, injected a rabbit's kidney with alcohol and was able to see an indistinct membrane in the glomerular loops. Fibers from the capsular basement membrane passed into the glomerular membrane. During digestion with pancreatin the epithelial and endothelial cells disappeared leaving a regularly striped fibrous membrane showing numerous fine pores. This confirmed the work of Drasch. Regaud and Policard, 1903, studying the kidneys of ophidians stained with alum hematoxylin, demonstrated a thin homogeneous membrane separating the endothelium from the synctyial epiglomerular layer. Hueter, 1908, stained the glomerular basement membrane with Ribbert's connective tissue stain. Gross, 1919, found that the membrane took a gray or brown color with Heidenhain's iron hematoxylin. Ohmori, 1921, obtained a very clear differentiation of the membrane with a rein blau-picric acid dye. Hung, 1922, was able to stain it distinctly if the tissue were boiled before fixation and later put through hematoxylin-eosin, Weigert's elastic tissue stain or Mallory's anilin blue. Krauspe, 1922, using Bielschowsky's method found the glomerular wall to consist of homogeneous gray or black membrane lined inside by endothelium and outside by epithelium. He saw the transition into Bowman's base-

ment membrane but could not detect the fine streaking mentioned by Rühle. Schäffer, 1927, noted the presence of the glomerular membrane but did not consider its structure to be fibrillar. von Moellendorff, 1927, demonstrated a homogeneous membrane with Heidenhain's azan-carmine and with his own eosin-methylene blue. Volterra, 1928, described the membrane as a continuous, argyrophil, net-like, reticular adventitia resting on endothelium but not formed by it and adherent to the pericytes. The processes which von Moellendorff assigned to the "deck" cells were, according to Volterra, the connective tissue fibrils of the membrane. Bargmann, 1929, described the glomerular membrane as a deep black or brown granular line when impregnated with silver.

Glomerular Connective Tissue: Key, in 1865, described star-shaped cells which he considered connective tissue between the glomerular loops. Ludwig, 1872, stated that the capillaries of the tuft had an investment of connective tissue. Klebs, 1863, and 1889, thought there were small numbers of such cells in all glomeruli. Toldt, 1874, saw central cells in the crevices of the tuft which were different from the epithelial cells. However, as the glomerulus developed and became spherical these connective tissue elements were no longer visible. In 1880, Waller confirmed Key's finding. In normal cat glomeruli he distinguished epithelium from "connective tissue corpuscles" which were situated between the lobes and stained with logwood after carmine and gelatin injections. He studied the glomeruli as a whole under a dissecting microscope and considered these cells to have a special affinity for vegetable coloring matter. Greenfield, 1880, also believed there were connective tissue cells within the glomerular tuft and Ribbert, 1879, described their nuclei as being small, spindle-shaped, and more intensely stained with carmine than the larger round finely granular epithelial nuclei. Opposing this view were Drasch, 1877, Langhans, 1885, Nauwerck 1886, Nussbaum, 1886, Hansemann, 1887, Orth, 1893, Mertz, 1918, and von Moellendorff, 1927. Nussbaum concluded that the connective tissue which he expected to find, in consideration of the embryological development, must have been resorbed. Herring, 1900, Mallory, 1914, and Vimtrup, 1928, have stated that some connective tissue persists around the hilum probably continuing into the glomerulus to bind the capillaries together.

With regard to the presence of reticulum within the tuft, John-

ston, 1899, believed these fibrils in Bowman's capsule were not reflected over the glomerulus. As the afferent vessel pierced the capsule, fibrils arose which penetrated the glomerulus, passing in all directions between its capillaries. These became less and less numerous as the periphery was approached. Luna, 1920, using the method of Achūcarro–del Río-Hortega, saw an occasional adventitial sheath in the glomerular capillaries. Corner, 1920, decided that the endothelium of the glomerular tuft was never provided with a reticulum. Krauspe, 1922, employing the Bielschowsky–Maresch technique, after a study of 150 human kidneys, could not find *gitterfasern* in the glomerular capillaries. Foot, 1925, decided that no reticulum was seen in the human renal tuft under ordinary circumstances. Allen, 1927, using silver impregnation concluded that occasional glomeruli showed reticulum fibrils in the tuft, usually near the pedicle. A few fibers radiated peripherally with no constant relation to reticulum cells or capillary endothelium. Bargmann, 1929, saw an argyrophil network deep in the capillary wall but he could not say to what it belonged.

Shape and Size of Glomerulus: Vimtrup, 1928, described the human malpighian body as oval with an average diameter of 200 microns as compared with Fahr's figure of 237 microns.

Mertz, 1918, found that the total number of cells in 10 to 15 micron sections through the greatest diameter of normal glomeruli was constant, and at most 145. Of these 3 to 30 were leucocytes, leaving 130 endothelial and epithelial. von Moellendorff, 1927, did not make a quantitative study but concluded that there were at least ten times as many epithelial as endothelial cells. Bargmann, 1929, made the same observation.

Development of the Glomerulus: According to Herring, 1900, and Huber, 1909, the malpighian body, convoluted tubules, Henle's loops, junctional tubules, connective tissue framework and capsule arise from the kidney blastema, a mass of cells formed from the intermediate cell mass. Blood vessels are partly of the same origin and partly branches from the renal artery. Malpighian bodies and their tubules begin to appear at the end of the second month. Each arises as a solid mass of cells which acquires a lumen and becomes S-shaped. The lower limb of the "S" becomes the malpighian body, the upper and middle limbs form the convoluted tubules. The convex side of the lower limb is formed by the epithelium which later lines

Bowman's capsule, the concave side by the epithelium which later covers the glomerulus. The glomerulus is at first non-vascular and is formed by a thickening of the epithelium rather than by an invagination. The capillaries form *in situ* from mesenchymal connective tissue cells close to the base of the glomerulus and are joined by a branch of the renal artery. The division of the glomerulus into lobes is due to the penetration of the epithelial cells between the capillary loops.

Summary

To summarize the present status of the histology of the normal glomerulus, it may be said:

1. The shape is oval and the diameter varies between 200 and 237 microns.

2. The capsule consists of a basement membrane lined by flat epithelial cells which become taller near the opening into the tubule. The structure of the membrane and its relation to the glomerular membrane are not agreed upon.

3. The wall of the glomerular loop contains a basement membrane whose porosity and fibrillar or homogeneous character are unsettled subjects.

4. Within the glomerular membrane are a few endothelial cells between which, boundaries have not been definitely established.

5. Outside the glomerular membrane is a layer of cells whose epithelial, adventitial and syncytial characters are controversial points.

6. The presence of reticular fibrils in the tuft beyond the wall of the vas afferens is indefinite as is also the existence there of connective tissue cells.

II. Methods Used and Results Obtained in this Study

Fixation of Tissues: In general the results were best following Zenker or Helly fixation. However, most of the tissues available for this work had already been fixed in 10 per cent formalin. Davidoff, 1928, described a method of taking nerve tissue from formalin to Zenker. This was tried on kidney tissue and was found to work very successfully. The technique may be applied to the tissue in blocks or to paraffin sections.

(*a*) For paraffin sections of formalin-fixed tissues.

1. One hour in ammonia (40 drops to 10 cc. of water).
2. One hour in running water.
3. One hour in Zenker's or Helly's fluid.
4. One hour in running water.

(*b*) For blocks of formalin-fixed tissue.

1. Two days in paraffin oven at 40° C in ammonia (40 drops to 100 cc. of water).
2. Twenty-four hours in running water.
3. Zenker twelve hours, or Helly five hours.
4. Twelve hours in running water.

Staining of Tissues: The following stains have been used: Hematoxylin and eosin, Mallory's eosin-methylene blue, methyl green-pyronin, Van Gieson, Weigert's elastic tissue stain, Ohmori's rein blau-picric acid, Mallory's anilin blue, Heidenhain's azan-carmine modification of Mallory's anilin blue, Lee-Brown modification of Mallory's anilin blue, Bielschowsky–Maresch, Bielschowsky–Perdrau (Bailey and Hiller), Bielschowsky–Ferguson, Bielschowsky–Foot, Hortega–Foot silver carbonate.

The technique for the three stains which were found to be the most useful is as follows:

(*a*) Mallory's anilin blue (Heidenhain's azan-carmine modification*).

1. 1 per cent azan-carmine G (1 gm. in 100 cc. of water, heat, cool, filter at room temperature and add 1 cc. glacial acetic acid). Thirty to forty minutes in this stain in the paraffin oven at 56° C.
2. Wash in water.
3. Differentiate in anilin alcohol (1 cc. anilin oil in 100 cc. 95 per cent alcohol). Watch under the microscope until the nuclei are red and the cytoplasm pale pink. This step requires from one to three minutes, depending on the thickness of the sections.
4. Remove anilin with acid alcohol, about one minute.
5. Three hours in 5 per cent phosphotungstic acid.
6. Wash quickly in water.
7. Three to six hours in:

anilin blue	0.5 gm.
orange G	2. gm.
glacial acetic acid	8. cc.
distilled water	100. cc.

Boil, cool and filter. Dilute one-half with water.

8. Wash in water.
9. Differentiate in *absolute alcohol, watching under the microscope.*
10. Xylol.
11. Balsam.

* Some changes were made in this technique so the directions do not correspond with the original as given by Heidenhain.

Nuclei orange-red, cytoplasm pink, connective tissue and reticulum blue, fibrin red.

(*b*) Ohmori's rein blau-picric acid.

1. Stain the nuclei with acid fuchsin or lithium carmine at least one-half hour.
2. One minute in rein blau-picric acid (to a concentrated aqueous solution of picric acid add a concentrated aqueous solution of rein blau until a dark green color appears).
3. Differentiate in absolute alcohol.
4. Xylol.
5. Balsam.

(*c*) Mallory's anilin blue, Lee-Brown modification.

1. Phosphomolybdic acid 1 per cent for thirty seconds.
2. Distilled water for 1 to 2 minutes.
3. Mallory's stain:

anilin blue	0.5 gm.	for 1 to 5 minutes
orange G	2. gm.	
phosphomolybdic acid	2. gm.	
distilled water	100. cc.	

4. Distilled water for 2 to 5 minutes.
5. Phosphomolybdic acid 1 per cent for 30 seconds.
6. Distilled water for 1 to 2 minutes.
7. Dehydrate, clear and mount.

The Capsular Basement Membrane: The outer layer of Bowman's capsule is composed of argyrophil fibrils which anastomose with the intertubular stroma of the kidney and with the reticular covering of the tubules. The inner layer of Bowman's capsule is an apparently homogeneous membrane which stains red with Van Gieson, blue with Ohmori and dark blue with the Mallory–Heidenhain azan-carmine. This structure is continuous with the glomerular and tubular basal membranes.

The Capsular Epithelium: These cells form a single layer completely lining the capsule. Their cell borders are distinct and as would be expected from their embryological origin they are directly continuous with the glomerular and tubular epithelium. Their nuclei are elongated, with finely dispersed chromatin and one or more large nucleoli.

Glomerular Epithelium: These are the cells which, in the embryo, form the concave side of the lower limb of the "S" (the convex side of the capsular space). During fetal life and early childhood the arrangement is columnar, this gradually becoming flattened in the adult.

These cells form a complete single layer of covering over the tips and crevices of the capillary tufts. At the hilum they are continuous with the capsular, and thus with the tubular epithelium. They lie outside the glomerular basement membrane which separates them from the endothelium. The arrangement is not syncytial and cell boundaries are easily seen. They are much more numerous than endothelium.

Each cell has abundant cytoplasm and a large oval nucleus situated far from the base. The chromatin is finely dispersed near the nuclear membrane and there are one or two nucleoli. The cytoplasm takes a basophilic tint with Mallory's eosin-methylene blue. The morphology is very similar to that of the capsular epithelium.

Glomerular Basement Membrane: The S-shaped mass of cells destined to form the glomerulus and its tubule is surrounded by a membrane. The portion of this structure covering the concavity of the lower limb of the "S" becomes the glomerular basement membrane. It is closely associated with the glomerular epithelium and is present before the glomerular capillaries appear.

In sections stained with anilin blue or with rein blau the membrane is a single deep blue line lying between endothelium and glomerular epithelium and forms the most striking portion of the glomerular capillary wall. It does not stain with any of the silver impregnation methods used.

For the most part its structure seems to be homogeneous. However, near the hilum there is sometimes a lamellated appearance. Whether the membrane is fibrillar or whether the extra fibrils belong to the connective tissue cells of the vascular pedicle has not yet been determined.

The vas afferens with its supporting framework passes well into the center of the glomerulus before it breaks up into capillaries. Its subendothelial connective tissue seems to fuse with the glomerular basement membrane.

The lumen of each glomerular branch of the afferent vessel is narrowed at fairly regular intervals with openings into numerous small capillary loops. At each of these orifices the basement membrane appears thickened and contracted while in all other portions of the capillary wall it is thin and quite uniform.

The continuity of the glomerular capsular and tubular membranes is evident in any fetal or adult kidney stained with anilin

blue or with rein blau. While differentiating these sections in absolute alcohol the glomerular basement membrane decolorizes before any other membrane or connective tissue in the kidney.

Glomerular Endothelium: These cells are continuous with the endothelium of the vas afferens and efferens and are situated inside the glomerular basement membrane. Their nuclei are widely separated and are much less numerous than those of the epithelium. A continuous layer of cytoplasm lining the membrane and connecting adjacent cells is rarely visible. The nuclei are small and elongated with a thick nuclear membrane and coarse, evenly distributed chromatin. The small amount of cytoplasm at each end of the nucleus makes the cell appear stretched and flattened against the basement membrane. The question of cell boundaries has not been investigated.

There is another type of cell in the wall of the capillary which is probably endothelial also. It is always situated inside the passageway between loops or adjacent to the orifice. In this way it lies next the thickened portions of the glomerular membrane and seems to be almost surrounded by it. This cell cannot be considered epithelial because it is inside the basement membrane. With the stains used in this study the nucleus cannot be distinguished from that of typical endothelium. Its cytoplasm is extremely minute, perhaps because of its position in the constricted portion of the capillary. There is a possibility that it is a connective tissue cell enclosed in the membrane. Further studies on this point are in progress.

Connective Tissue Cells: These accompany the afferent vessel almost to the center of the glomerulus and are always visible in sections through the greatest glomerular diameter. Reticulum argyrophil fibrils also enter at the hilum, pass into the glomerulus and end in finer fibrils or very small clusters. They do not accompany the basement membrane of the capillary loops but seem rather to be associated with the afferent vessel.

Summary

The following conclusions have been reached after studying oil immersion fields of tissues stained as described previously.

1. The glomerular, capsular and tubular basement membranes are continuous and stain red with Van Gieson, blue with Ohmori and

dark blue with Mallory–Heidenhain azan-carmine. The capsule and tubules have an additional external, reticular, argyrophil covering. The glomerular basement membrane is thinner than that of the capsule and tubules. Pores have not been demonstrated, nor any elastic fiber content.

2. The glomerular, capsular and tubular epithelium are continuous. The glomerular epithelial cells have abundant cytoplasm and large vesicular nuclei with one or more prominent nucleoli. These are the only cells which lie outside the glomerular basement membrane. They form complete covering for the loops following closely both tips and crevices. They are arranged in a single layer and not in a syncytium.

3. The glomerular endothelium lies inside the basement membrane and therefore cannot be confused with epithelium. The cytoplasm is very small in amount and the nuclei have a thick nuclear membrane enclosing much coarse chromatin. Endothelial cells are much less numerous than epithelial.

There are cells, probably endothelial, which lie inside the glomerular basement membrane but are partially surrounded by it. Their nuclei seem to be identical with those of typical endothelium and they have very little cytoplasm. Their situation at the orifices between loops makes examination very difficult. Further study to decide their nature is in progress.

4. Connective tissue cells and reticulum fibrils pass in at the hilum accompanying the vas afferens until it subdivides near the center of the glomerulus.

BIBLIOGRAPHY

See following paper for bibliography.

DESCRIPTION OF PLATE

Plate 104

Fig. 1. Diagram of section through greatest diameter of a normal glomerulus. C. tub. = convoluted tubule; aff. a. = afferent artery; c.t. = connective tissue; c.b.m. = capsular basement membrane; c.ep. = capsular epithelium; gl.ep. = glomerular epithelium; gl.b.m. = glomerular basement membrane; end. = endothelium; end. l. = type of endothelial cell found at passageway between loops; r.b.c. = red blood cell; l. = leucocyte; t. = thickening of glomerular basement membrane often seen at orifice into another loop.

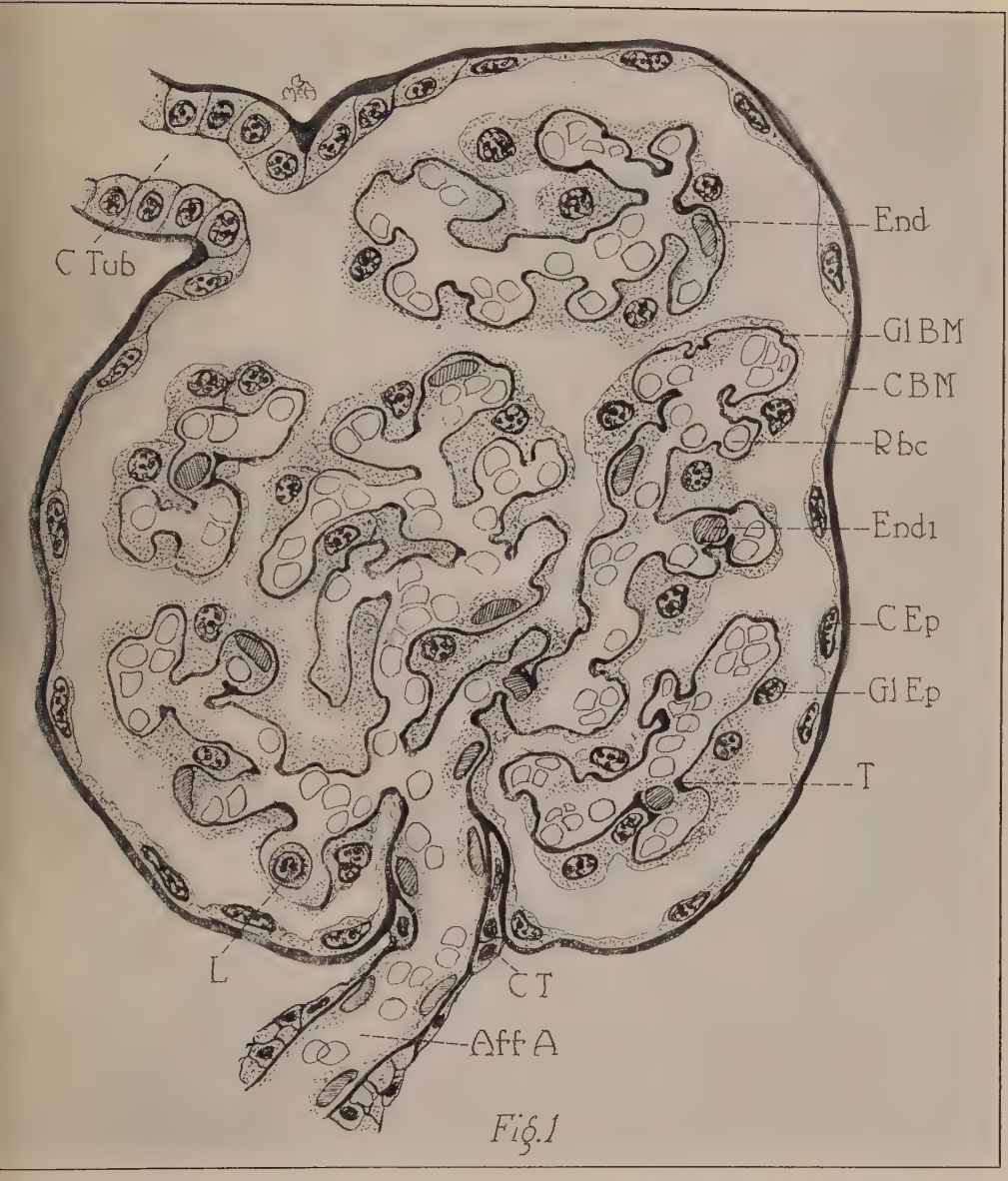

Fig. 1

THE CYTOLOGICAL CHANGES OCCURRING IN THE GLOMERULUS OF CLINICAL GLOMERULONEPHRITIS *

LEONE MCGREGOR, PH.D., M.D.

(*From the Department of Pathology, University of Minnesota, Minneapolis, Minn.*)

INTRODUCTION

The object of this paper is to review the literature and to present some new facts regarding the cytological changes occurring in the glomerulus of clinical glomerulonephritis, acute and chronic.

I. REVIEW OF LITERATURE

Richard Bright in 1827 considered the renal lesions in nephritis to be of an inflammatory character. The work of Christison, 1839, Rayer, 1840, Johnson, 1847 and 1850, Frerichs, 1851, Virchow, 1852, and Wilks, 1853, directed attention toward the tubular changes of fatty degeneration, desquamation and proliferation. However in 1850, Johnson observed changes in the glomerular capillary walls in chronic renal disease. From that time on the glomeruli as well as the tubules were studied.

Glomerular Endothelial Reactions: Since most of the very early investigators were convinced of the interstitial and tubular nature of nephritis, very few studies were made of glomerular endothelium. Johnson, 1873, noted that the nuclei in the walls of the glomerular capillaries were abnormally conspicuous. Langhans, 1879, was the first to call attention to endothelial proliferation. Hortoles, 1881, describing a case of scarlet fever, believed the multiplication of the endothelial cells was due to their reversion to an embryonic state. Friedländer, 1883, saw proliferation of "the endothelial tube." Aufrecht, 1886, found a swelling but no increase in number of these cells. In 1885 Langhans' detailed description of the glomerular capillaries in acute nephritis supported what he believed to be the essential lesion, an almost complete occlusion of the loops by endothelial proliferation. Welch, 1886, corroborated this statement. Nauwerck,

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1886, saw endothelial cells with several nuclei and supposed their multiplication was so extensive that they were cast off into the lumen. Ribbert, 1879, and Hansemann, 1887, because of the scarcity of endothelial nuclei in normal glomeruli could not believe they were the source of proliferation in nephritis. Klebs, 1889, differing from his earlier publication, stressed the swelling of endothelium as part of the lesion. Sorenson, 1891, reached the same conclusion. Councilman, 1897, reviewing forty-nine cases of acute and subacute nephritis, confirmed Langhans in the conclusion that most of the nuclear increase was endothelial. Herring, 1900, made a similar observation. Löhlein, 1910, described a proliferation and "net-like arrangement of cellular elements of the endothelial type." Kaufmann, 1911, saw a marked increase in endothelium with desquamation into the capillary lumen. Gaskell, 1911, observed a homogeneous swelling of the capillary wall cells with subsequent fatty degeneration. Aschoff, 1913, Fahr, 1914, Mallory, 1914, MacCallum, 1916, Dietrich, 1917, and Jungman, 1917, all explained the narrowed lumina of the capillary loops as due to endothelial proliferation. Mertz, 1918, in his quantitative study, concluded that between the ninth and twenty-first days of nephritis the endothelial cells at least doubled in number, their increase being proportional to the severity of the disease. Since he believed the number of glomerular epithelial cells to be constant, his figures for endothelial increase are inexact. Herxheimer, 1916, and 1918, in a comprehensive review of war nephritis, stated that the first morphological change was growth of endothelium. Gross, 1919, confirmed Kaufmann as to the proliferation and desquamation of these cells. Mallory, 1914, Ohmori, 1921, Bell and Hartzell, 1922, Munk, 1925, Kuczynski and Dosquet, 1926, Elwyn, 1926, and Hückel, 1928, all described varying degrees of increase in endothelium. MacCallum, 1921, said "the glomerular tufts become greatly enlarged through the appearance of quantities of cells in the lumina of the capillaries, which are not polymorphonuclear leucocytes, but look more like endothelial cells. At least they are mononuclear cells with indistinct outline, so fused together that their origin can hardly be made out." Fahr, 1925, observed such marked proliferation and desquamation that a two to three-celled layer was formed in the capillaries.

Mallory, 1914, Herxheimer, 1916, Gross, 1919, and Elwyn, 1926, have reported mitoses in glomerular endothelial cells.

Intracapillary Leucocytes: The opinion has been uniformly held that a part of the glomerular cell increase was polymorphonuclear. However, Buhl, 1878, and Waller, 1880, decided the leucocyte accumulation was the outstanding feature. Langhans, 1885, stated that leucocytes helped to occlude the glomerular capillaries, their cytoplasm fusing with that of the proliferating endothelium. Cornil, 1879, and Hansemann, 1887, believed the so-called endothelial multiplication was merely an aggregation of leucocytes. Obrzut, 1888, and Ribbert, 1888, both agreed with Buhl and Waller that the leucocytes could later form connective tissue. Gräff, 1916, Herxheimer, 1916 and 1918, and Mertz, 1918, studied in detail the polymorphonuclear exudate. Mertz concluded that in very early stages these cells were increased up to five times their normal number. Aschoff had pointed out that leucocytes in glomeruli might not be due to inflammation, but merely a condition seen in the agonal stage in circulatory disease. After a study of control cases Herxheimer disagreed. Mallory, 1914, called attention to the participation of endothelial leucocytes (large mononuclears or monocytes) in the exudate in slowly progressive cases. He suggested that some wandered in from the blood stream, while others were desquamated endothelium. Bell and Hartzell, 1922, considered the leucocytic reaction polymorphonuclear in type.

Glomerular Intracapillary Network: Cellular and non-cellular types of network within the glomerular loops have been described. In 1849, Rokitansky explained the interference with the glomerular circulation on the basis of a fibrino-albuminous deposit within the capillaries. According to Langhans, 1885, the growing endothelium formed a reticulum within the loops and led to closure of the lumina. To Hansemann, 1887, this conception was erroneous and was apparent only in very thick sections. Klebs, 1889, described obstruction of the capillaries by a syncytium composed of endothelium, leucocytes and masses which stained red with eosin. Thrombotic occlusion of the loops by endothelial cells and leucocytes or by granular masses was seen by Friedländer, 1883, and Nauwerck, 1886. Böhm, 1897, and Kaufmann, 1911, also described an accumulation of fine granular detritus coming from endothelium and blood corpuscles. Fibrin excretion was prominent in the scarlet fever cases studied by Reichel, 1905. Herxheimer, 1916, believed leucocytes were mechanically held in the loops due to injury of the endothelium. He decided coagulated blood serum was the source of the cytoplas-

mic network described by Aufrecht, 1894, as coming from red blood cells. Mallory, 1914, described fibrin in the loops in the form of threads, a network or solid masses. Löhlein, 1910, and Rochs, 1918, observed a coagulated cytoplasmic meshwork within the vessel lumina. Gross, 1919, found free endothelial cells within the network which stained with cresyl violet and Heidenhain's pale blue but not with Van Gieson or Weigert. Ohmori, 1921, saw a deposition of fibrin flakes on the inside of the glomerular basement membrane. These increased in amount, became confluent and crossed the loops. MacCallum, 1916, ascribed the capillary occlusion to the proliferating endothelium and to indefinite foamy masses of cytoplasm. Aschoff, 1921, mentioned the presence of fibrin and nuclei in the cytoplasmic glomerular network. Excessive endothelial cytoplasm alone obstructed the lumen in a case described by Fahr, 1925.

Glomerular Epithelium: Klein, 1877, was the first to mention an increase in nuclei of cells "probably epithelial" covering the tuft. Langhans, 1879, saw such swelling of the cytoplasm and nuclei of these cells that they were attached only by narrow pedicles to the loop wall. When the increase in size progressed far enough they were desquamated in large numbers. Weigert, 1879, and Ziegler, 1879, observed swelling, degeneration and destruction of glomerular epithelium as one of the earliest changes in acute nephritis. Greenfield, 1880, and Saundry, 1880, found proliferation of epithelium, which they called endothelium because they considered it comparable to the peritoneal and pleural lining cells. Litten, 1878, and Dunin, 1883, believed the degenerating epithelium liberated chemicals injurious to the vessel walls and causing inflammation, although Dunin also stated that vascular changes could occur independently of epithelial disease. Aufrecht, 1886 and 1894, described swelling but not proliferation of glomerular epithelium. Kiener and Kelsch, 1882, Cornil and Brault, 1883, Nauwerck, 1886, and Klebs, 1889, all saw multiplication, degeneration, necrosis and desquamation of these cells until the capillary loops were bare. Welch, 1886, and Ribbert, 1888, noted these same epithelial changes. Hansemann, 1887, explained the albuminuria as due to the nakedness of the capillary wall following the desquamation. Ernst, 1893; thought fibrin was able to pass through the glomerular loop after the death of the epithelium. Orth, 1893, and Böhm, 1897, described degeneration of these cells. Herring, 1900, concluded glomerular epithelium was

highly organized and therefore easily injured. Herxheimer, 1909, agreed with Aufrecht, 1886, and with Engel, 1901, that the primary injury in glomerulonephritis is epithelial. Löhlein, 1910, found slight swelling of the epithelium in early stages which passed on to desquamation in severe cases. Kaufmann, 1911, saw both proliferative and degenerative changes. Herxheimer, 1916, observed swelling and the formation of giant cells in this epithelium. Mertz, 1918, on the other hand, decided that the number of epithelial cells was a constant which did not increase. Gross, 1919, felt that endothelial proliferation preceded epithelial degeneration. Mitoses were mentioned in the glomerular epithelium by Mallory, 1914, MacCallum, 1916, Fahr, 1925, Munk, 1925, and Kuczynski and Dosquet, 1926; all showed illustrations of the changes occurring in these cells.

Hyaline granular degeneration of glomerular epithelium similar to that commonly seen in convoluted tubules has been described by Posner, 1880, Aufrecht, 1886, Kaufmann, 1911, Mallory, 1914, Herxheimer, 1916, Gross, 1919, Aschoff, 1921, Fahr, 1925, and Kuczynski and Dosquet, 1926.

Glomerular Connective Tissue Proliferation: Klebs, 1863, stated that in glomerulonephritis the inflammation was situated between the coils of the glomerulus in the small amount of connective tissue there. By 1889, his opinion was modified to the extent that he no longer considered connective tissue proliferation a conspicuous feature. Waller, 1880, confirmed Klebs' original statement because the first nuclear increase occurred between the loops and later progressed toward the tips. Greenfield, 1880, and Böhm, 1897, described a proliferation of connective tissue cells around the vas and between the glomerular loops. Councilman, 1897, and Herring, 1900, thought a few of the new cells were of connective tissue origin. Mallory, 1914, stated that the fibroblasts forming the supporting framework of the capillaries were stimulated to proliferation.

Capsular Epithelial Proliferation: Swelling and proliferation of these cells were first described by Langhans, 1879, Ziegler, 1879, and Greenfield, 1880. The latter two believed the cells to be endothelial with a reaction similar to that of the serous membranes. Saundry, 1880, von Leyden, 1881, Aufrecht, 1886 and 1894, Nauwerck, 1886, Welch, 1886, Ribbert, 1888, Klebs, 1889, Orth, 1893, von Kahlden, 1894, Herring, 1900, Herxheimer, 1909, Löhlein, 1910, Gaskell, 1911, Mallory, 1914, MacCallum, 1916, Aschoff, 1921, Bell and Hartzell,

1922, Fahr, 1925, and Munk, 1925, all confirmed the work of their predecessors on this point.

Hyaline granular degeneration of capsular epithelium has been reported by Aufrecht, 1894, Mallory, 1914, and Fahr, 1925. Mitoses were seen in these cells by Mallory, 1914.

Randerath, 1929, demonstrated anatomically the presence of a pericapsular capillary net. He suggested that bacteria in these capillaries causing an increased permeability of the capsular basement membrane could stimulate the capsular epithelium to proliferation.

Crescent Formation: Langhans, 1879, Nauwerck, 1886, and Fahr, 1925, decided the chief source of the cells in the crescent was desquamated glomerular epithelium. Ziegler, 1879, Greenfield, 1880, Leyden, 1881, Dunin, 1883, and MacCallum, 1916, have ascribed the origin of crescents to capsular epithelium. Saundry, 1880, Welch, 1886, Hansemann, 1887, Orth, 1893, Engel, 1901, Löhlein, 1910, Kaufmann, 1911, Mallory, 1914, Herxheimer, 1916, Aschoff, 1921, and Munk, 1925, have all stated that both capsular and desquamated glomerular epithelium take part in crescent formation. Waller, 1880, and Obrzut, 1888, suggested that emigrated leucocytes changing into connective tissue produced the crescent.

The presence of fibrin in the capsular space and crescent has been reported many times. It was described as a conspicuous feature by Ernst, 1893, Engel, 1901, Löhlein, 1910, Kaufmann, 1911, Mallory, 1914, MacCallum, 1916, Ohmori, 1921, Aschoff, 1921, and Fahr, 1925. Ernst saw a fibrin net between the cells of the crescent and extending into the convoluted tubule. The descriptions of Mallory, Aschoff and MacCallum were very similar. According to Engel, the source of the fibrin was either the blood plasma or the degenerated epithelium. Löhlein explained the fibrin as forming from blood extravasated into the capsular space. Fahr disagreed with this view and described it as an inflammatory excretion. Herxheimer, 1916 and 1918, found no intracapsular fibrin in the war nephritis cases.

Herxheimer described hyaline bodies in the crescent which were not fibrin, which stained yellow with Van Gieson, and which were sometimes inside the cytoplasm of growing epithelial cells. MacCallum also saw hyaline masses in contact with the epithelium and thought they arose from fusion of disintegrating red blood corpuscles which had passed through the capillary wall.

Almost all workers have described coagulated albumin, red blood

cells, polymorphonuclears, desquamated disintegrating glomerular epithelium and fat in the capsular space or in the crescent. Langhans, Gräff and Herxheimer have seen leucocytes passing through the wall of the glomerular loops.

In an explanation of the connective tissue organization of the crescent, Mallory, 1914, stated that fibrin in the capsular space stimulated fibroblasts in the tuft and capsule wherever it touched them. They proliferated and organized until the fibrin was replaced by collagen. MacCallum, 1916, believed the proliferating epithelial cells brought with them a supporting framework of connective tissue.

Changes in Permeability of Glomerular Capillary Wall: The large amount of exudate in the tubules and capsular spaces in nephritis has suggested an increased permeability of the glomerular capillary walls. No one has been able to demonstrate stomata or other morphological evidence of this process. Toynbee, 1846, and Thoma, 1877, showed experimentally that in normal kidneys, injections of a calcium solution and Berlin blue at a pressure of 50 mm. Hg. very rarely went through the loops into the capsular space. On the other hand, in nephritic kidneys, the more severe the disease the more material passed through the capillary walls. Nauwerck, 1886, Hansemann, 1887, and Engel, 1901, believed the increased permeability was due to the degeneration and desquamation of the glomerular epithelium.

Changes in the Glomerular Basement Membrane: Ohmori, 1921, was the only writer to note changes in the basement membrane in nephritis. He believed it could be mechanically compressed and physically or chemically altered. He observed that it was often attached to the intracapillary hyaline masses seen in old chronic glomerulonephritis. Within the loops fibrin was deposited on the membrane and finally fused with it.

Changes in the Vas Afferens: Langhans, 1879, Nauwerck, 1886, Reichel, 1905, Löhlein, 1910, Mallory, 1914, MacCallum, 1916, Herxheimer, 1918, Gross, 1919, Aschoff, 1921, Bell and Hartzell, 1922, and Fahr, 1925, have all concluded that glomerulonephritis is a primary inflammation in the capillaries of the glomeruli. Other workers have believed the primary change to take place in the vas afferens with a secondary inflammation of the glomeruli. Klein, 1877, after finding a multiplication of muscle and connective tissue nuclei in the coat of the afferent vessel, believed the thickening of

its wall tended to shut off the circulation to the glomeruli. Hlava and Thomayer, 1882, were of the opinion that a small round-celled infiltration in the interstitial tissue around the afferent and efferent vessels compressed their lumina. Aufrecht, 1886 and 1894, confirmed the findings of Klein. Herxheimer, 1918, felt that Aufrecht's conclusions were due to the fact that he was not studying the earliest stages of the disease. Volhard and Fahr, 1914, decided that a spasm of the afferent vessel led to ischemia of the glomerulus and secondary inflammatory processes there. Kuczynski and Dosquet, 1926, described a severe edema in the region of the afferent vessel with occlusion of its lumen in early acute nephritis. The edema stimulated resting cells of the adjacent connective tissue until they wandered into the wall of the vas and formed new cells around the hilum. Hückel, 1928, disagreed with Kuczynski and Dosquet after studying a very early acute case, a woman aged 39 years, who died of uremia thirty hours after the onset of symptoms. He was unable to find swelling or edema at the vascular hilum in more than a very few glomeruli. All workers have agreed that there is a great deal of arteriolar disease in the advanced stages of glomerulonephritis.

Hyalinization of the Glomerulus: Klebs, 1863, concluded that the connective tissue cells between the loops as well as emigrated leucocytes changed into a hyaline substance which later became organized. Klein, 1877, believed there was a primary arteriolar lesion which led to a hyaline degeneration and fibrosis of the glomerulus and its capsule. Thoma, 1877, described a connective tissue change of the vascular loops of the glomerulus. The walls thickened and acquired a homogeneous appearance while the capsule formed concentric layers of fibrous cellular connective tissue. In one type of atrophy the loops did not completely disappear while in the other type they were replaced by concentric layers of connective tissue. Ziegler, 1879, described two types of atrophy, one due to the proliferation and pressure of a crescent on the tuft, the other due to obliteration of the vas afferens. Greenfield, 1880, believed proliferation of glomerular connective tissue cells as well as the crescent led to fibrosis of the glomerulus. Waller, 1880, concluded that the hyalinization of the malpighian body was due to a connective tissue transformation of leucocytes which had migrated into the capsular space. The connective tissue cells normally present between the loops proliferated and assisted in the process. Dunin, 1883, found

three types of contracted glomeruli due to Bright's disease. The types differed in size, fusion or non-fusion of glomerulus and capsule, and partial or total loss of the original lobular structure. Friedländer, 1883, believed the walls of the capillary loops thickened until the lumina were occluded. Nauwerck, 1886, described a hyaline thickening (similar to amyloid degeneration) of the loop walls, plus an occlusion of the capillaries by leucocytes, endothelium and granular masses. Böhm, 1897, observed pericapsular and hilum connective tissue proliferation causing atrophy of glomeruli. Goemans, 1899, described a connective tissue proliferation in the glomerulus and in the capsule. Engel, 1901, believed that organization of the fibrin in the exudate was the source of the connective tissue growth of loops and capsule. Tschistowitsch, 1903, saw pericapsular connective tissue growing in through the capsule at various points and also from the hilum. In some cases swelling of the capsular basement membrane and collapse of the capillaries were the sources of the capsular and glomerular hyalin. Löhlein, 1910, described a homogeneous thickening and hyalinization of the loop walls and fusion of the whole with the capsule. Mallory, 1914, found that a proliferation of fibroblasts in the tuft produced more collagen which contracted toward the hilum until the capillaries were obliterated. This left a non-vascular mass of hyaline connective tissue. Krauspe, 1922, considered glomerular hyaline to be different chemically from capsular hyalin, as shown by their reactions to the Van Gieson stain. He concluded that the two hyaline materials were in a different colloid state. Calcification of the hyaline glomerulus has been reported by several authors.

Summary

1. The opinion has been quite uniformly held that there is a marked proliferation of glomerular endothelium in the acute stages of nephritis.

2. The leucocytic exudate has usually been described as polymorphonuclear. The origin of the mononuclear cells within the capillaries is doubtful, whether from the blood stream or from freed endothelium.

3. Protoplasmic, albuminous and fibrinous types of intracapillary network have been reported. There has been no agreement on this point.

4. Proliferation, degeneration and desquamation of glomerular epithelium have been established as one part of the morphological change.

5. There has been no uniform explanation of the origin and organization of the cells of the crescent.

6. Changes in the vas afferens have been considered for the most part as secondary to the inflammation in the glomerulus.

7. Very little attention has been directed toward alterations in the glomerular basement membrane.

8. The process of hyalinization of glomeruli in nephritis has not been described in detail.

II. Results Obtained from a Microscopic Study of Sixty Cases of Clinical Glomerulonephritis

Sections were stained as described in the previous paper. The descriptions below apply to the Mallory–Heidenhain azan-carmine stain unless otherwise specified.

Glomerular Epithelium: These cells covering the outside of the loops are among the first to react in nephritis. There is a cytoplasmic increase up to two or three times the original amount. With the azan-carmine it appears pale purple and denser than the capsular epithelium. With Mallory's eosin-methylene blue stain the periphery of each cell is basophilic, while the center is acidophilic. The nuclei enlarge and often become indented as if preparing for amitosis. Many cells contain two identical nuclei lying side by side. Mitosis has not been observed. The nuclear membrane is very delicate, and except for one to three nucleoli the chromatin is diffusely distributed in very minute particles. The nuclei are always situated in the periphery of the cell as far from the glomerular basement membrane as possible. Only a thin rim of cytoplasm separates the nucleus from the capsular space. Whether by mitotic or amitotic division, these cells are soon increased to two or three times their original number. This swelling and proliferation occur around every loop in every glomerulus and completely fill in the space normally present between lobules. When cut through the hilum and the junction with the convoluted tubule, the glomerulus appears oval in shape. Often one lobule with its enormous epithelial cells projects into and almost occludes the tubule.

The next change in the epithelium seems to be degenerative. Small hyaline granules and vacuoles appear in the cytoplasm of some of the cells. These bodies stain deep blue and are similar to the hyaline granules so commonly observed in the convoluted tubules in amyloid disease. At the same time the attachment of many of the cells to the loop wall becomes so narrow that it resembles a pedicle (see Fig. 3). This keeps the bodies suspended in the capsular space. The cells are so numerous that in spite of the narrow pedicles the loops are not left bare.

The hyaline granular degeneration may increase until the entire cell is filled. The nucleus may or may not disintegrate. In any case a certain number of cells are desquamated. They lie in the capsular space between fibrin clumps or applied to the capsular epithelium or on the inner surface of a crescent. When the pedunculated shape is retained the glomerular origin is evident, and even when the cell rounds off, its cytoplasm is much denser and more darkly staining than that of the capsular epithelium.

Desquamation is not extensive and soon ceases. Hyaline granular and vacuolar degeneration may continue for some time. As the intracapillary lesions progress, the swelling and proliferation of the glomerular epithelium either stay at a standstill or regress. The cases showing the most desquamation are left with very little epithelium. Bare loops have not been observed.

There is a reason for believing that the degeneration follows proliferation. It is the observation that the earliest intracapillary changes are accompanied by proliferation of epithelium, while the more advanced intracapillary changes seem to be accompanied by degeneration and desquamation.

Glomerular Basement Membrane: During the early stages of glomerulonephritis the changes in the glomerular membrane are rather insignificant. In those glomeruli showing extensive hemorrhage into the capsular space there is much wrinkling and indentation of the membrane, probably due to the external pressure. A careful search was made for pores or tears in the membrane through which the red and white blood corpuscles might have escaped. No openings were found even when the extravasation was so recent that it was not hemolyzed.

Glomerular Endothelium: The endothelial cells lie inside the glomerular basement membrane. They react by swelling and prolifera-

tion so that very early in nephritis there is a complete cytoplasmic wall lining the loops. As many as five mitoses have been seen in one section of a glomerulus. Multiplication continues until there are two or three layers of these cells in each loop (see Fig. 2). The nuclei are now very large and usually elongated due to crowding. Many are deeply indented, the chromatin is coarse, and there may be nucleoli. There is a large amount of cytoplasm, but the outline of each cell is irregular and rather frayed as if it had recently been attached. It appears as if many are desquamated into the lumen, where they are held in a meshwork of leucocytes, fibrin, and hyaline fibers (to be described later). At least in favor of desquamation is the fact that these large mononuclear cells seen in the lumen of the loop are identical morphologically with the adjacent endothelium.

Occasionally pyknosis and karyorrhexis are seen in endothelial cells. This condition is difficult to distinguish from the disintegrating polymorphonuclears, which always seem to stick to the capillary wall.

Glomerular Intracapillary Leucocytes: Polymorphonuclears are not as conspicuous in this series of cases as in those of other authors. In the very early acute cases there are from one to two in each loop, that is, they are about one-third to one-half as numerous as the endothelial cells. When pyknotic they are seen adhering to the capillary wall, while when healthy they remain out in the lumen between the mononuclears. A few eosinophils but no lymphocytes have been observed.

The lumina of the glomerular capillaries are occluded for the most part by mononuclear cells. Some of these may be monocytes. With the stains used in this study neither their nuclei nor their cytoplasm could be distinguished from that of the proliferating endothelium. The numerous mitoses, the adjacent position of these cells and their similar morphology, all point to their endothelial origin. They occasionally appear to be free cells, but usually lie in layers or in a mass. Sometimes the lumen is completely stopped up with cells, but usually there is a narrow slit toward one side of the loop where the circulation may continue.

Neither polymorphonuclears nor mononuclears were ever seen passing through the glomerular membrane. There was no evidence of phagocytosis of disintegrating cell fragments.

Intracapillary Fibrin and Débris: Fibrin in clumps, rods or threads

is occasionally seen in a few glomerular loops. It usually lies close to the capillary wall and takes a bright red color with the Mallory–Heidenhain technique. Coagulated albumin, disintegrating red blood cells and pyknotic polymorphonuclears may be found in the lumina of the loops.

Intracapillary Hyaline Fibers: (Figs. 2 and 3.) It is these hitherto undescribed fibers which eventually lead to hyalinization of the glomerulus. It is believed that they are a typical finding in clinical glomerulonephritis. They were found in all but two of our sixty cases.

These hyaline fibers are present in the earliest stages as fine lines lying between the proliferating endothelial cells and leucocytes. Knots are numerous at intersection points. The fibers with their knots stain deep blue (exactly the color of the glomerular basement membrane) with Mallory–Heidenhain, red with Van Gieson, not at all with silver and are practically invisible with Mallory's eosin-methylene blue and with hematoxylin-eosin. They seem to be attached at one end to the glomerular basement membrane. They always lie along the edge of the cytoplasm of the intracapillary cells. The fibers form a network within the capillary which helps to hold in place the leucocytes and endothelial cells. The occurrence of the fibers is diffuse in most of the loops of almost all glomeruli. They are not seen except in association with endothelial proliferation. Therefore, they are inconspicuous in (1) those cases which show only crescent formation with very little intracapillary lesion and (2) in those glomeruli which are compressed by the hemorrhage in the capsular space.

As the disease progresses, these fibers increase in diameter and in number until they form a dense meshwork enclosing the newly formed endothelial cells. In the earlier stages a few pyknotic polymorphonuclears may be included, but as the fiber meshwork thickens only mononuclear cells remain. The polymorphonuclears have disintegrated. The fibers remain attached to the basement membrane in many places. Usually a narrow channel is left along one side of the loop where the circulation continues. By this time the fibers are much thicker in caliber than the basement membrane of the loop they occupy (Figs. 4 and 5).

The origin of the hyaline fibers is not as yet determined. The possibilities would seem to be: (1) that they are branches or offshoots

from the glomerular basement membrane; (2) that they are fibrin threads which become transformed into collagen; (3) that they are products of disintegrated red or white blood cells; and (4) that they are reticulum or collagen fibers formed intercellularly from the constituents of the blood stream under the influence of a secretion from the proliferating endothelium. In this latter case the disintegrating polymorphonuclears might have some influence.

It should be mentioned that these fibers are believed to be real and not artefacts, because they have not been seen in the sections of numerous normal control kidneys.

The points in favor of the first possibility are the facts that the fibers and membrane are usually attached to one another and that their staining reactions are similar. The demonstration of a fibrillar character in the membrane would give support to this theory.

The work of Baitsell, 1915, 1916, 1917, and 1921, would point toward the feasibility of the second theory. He has shown in living cultures and in wound healing the transformation of a fibrin net into fibrous tissue either by mechanical factors alone or by the influence of living cells. Foot, 1921, believed collagen fibers were a product of the transformation of fibrin, but in 1925 concluded that this was erroneous and that the two were independent of each other. He disagreed with White and Smith, 1924, who believed that fibrin in the center of a tubercle was converted to reticulum by the agency of endothelium. Foot, 1925, demonstrated a reticular network in tubercles one week old before the fibrin appeared. He believes fibrin plays no part in the formation of reticulum and that reticulum is converted directly into collagen. However, the fibers in glomerulonephritis stain blue, while the fibrin is red and both are present at the same time. When first formed, the hyaline fibers are fine sharp lines while the fibrin is usually in coarser rods or clumps. If the fibers do come from fibrin, the colloid state of the latter must be considerably altered and with that its staining reaction and morphology.

With regard to the third possibility, it has been shown by Foot, 1927, that reticulum fibrils in hanging-drop tissue cultures of blood can come from the disintegration of erythrocytes and a rearrangement of the reticulum they contain. He does not suggest that this occurs frequently in the body but that other cells might have an intracellular network which would be deposited by lysis. That red blood cells are not the origin of the intracapillary fibers in glomerulo-

nephritis can be shown with silver impregnation. The disintegrating red cells and their fragments in the convoluted tubules take the silver stain, while in the same section the intracapillary fibers do not.

The fourth possible origin of the fibers involves a discussion of the relation between reticulum and collagen. Russakoff, 1908, first described the transformation of reticulum into collagen. Miller, 1924 and 1927, considered reticulum as precollagen, for in tubercles where there was a tendency to heal, the former became converted into the latter. Baitsell and Mason, 1926, and Baitsell, 1927, have also made a study of the origin of fibrous tissue in tuberculous inflammation. They believe there is a transformation of the intercellular exudate into fibrils and fibrous tissue. "This does not preclude the possibility that the movement of the cells through the exudate may not, by means of purely mechanical factors, change the structure of the fibrous material, or that the secretions of various types of cells may so influence the fibers that their chemical composition becomes changed." The very detailed work of Orsós, 1926, shows that reticulum is only changed collagen and transitions in either direction from one to the other can be seen in normal lymph nodes. He states, and his illustrations show that "excluding certain pathological conditions" reticulum or collagen formation is intracellular. This substance is first seen inside the cytoplasm of the cell as blue-staining elements with Mallory's anilin blue. However, the intracapillary fibers in glomerulonephritis with this same stain seem to be intercellular rather than intracellular. Mallory and Parker, 1927, in a study of reticulum and collagen, concluded that all collagen is produced by fibroblasts, that endothelial cells do not produce an intercellular substance and that reticulum is collagen in a separated form. In order to fit into this theory the intracapillary mononuclear cells in nephritis would have to be considered fibroblasts. Certainly there are no connective tissue cells out in the tips of the loops according to any authority. Yet fiber production is just as plentiful there. According to this study there are only endothelial cells inside the basement membrane of a loop. Therefore the fibroblasts would have to come from endothelium. But the new cells are morphologically more like endothelium than like fibroblasts. The nuclear membrane is thick and often indented, and the chromatin is arranged in rather coarse clumps. These cells do seem to assist in the formation of fibers which ultimately are indistinguishable from collagen. Per-

haps endothelium and its derivatives can produce fibers. Maximow, 1928, studied the formation of reticulum and collagen in cultures of connective tissue and also of blood leucocytes. The process is to be thought of as a crystallization or precipitation. The colloidal sol surrounding the fibroblasts is changed into a gel under the influence of a chemical excretion from the cell bodies. Fiber formation is intercellular. The fibers first appear in the form of an argyrophil network, which is identical with reticulum. With increase in amount, they become parallel and arrange themselves in wavy bundles. At this stage they lose their argyrophil properties and stain blue with anilin blue or red with Van Gieson. Often the fibrin threads form a supporting framework for the fibers, but no direct transformation from one to the other was observed. Above all, the fibers develop extracellularly. The intracapillary glomerular fibers are similar to Maximow's in that they form extracellularly and perhaps from the constituents of the blood. Maximow would explain the newly formed endothelial cells as fibroblasts. On the other hand, the hyaline fibers do not stain with silver even in the early stages but seem to be closely allied to collagen from the beginning.

Thus far in this study it would seem that the hyaline intracapillary fibers in nephritis either come from the glomerular basement membrane or arise intercellularly in a manner comparable to those described by Miller, 1924 and 1927, Baitsell and Mason, 1926, Baitsell, 1927, Mallory and Parker, 1927, and Maximow, 1928. If they arise from the constituents of the blood stream, it must take place under the influence of the proliferating endothelium and disintegrating leucocytes. The fact that the fibers do not stain with silver may be due to lack of sufficiently early stages of nephritis to study.

Glomerular Connective Tissue: As the glomerulus increases in size in the early stages of nephritis, it extends farther down on the vas afferens. Thus more connective tissue cells appear to follow the afferent vessel into the glomerulus. In addition to this apparent increase there is a slight proliferation of these cells. Their location prevents confusion with the intracapillary cellular proliferation. In no case was there seen sufficient proliferation of these cells to occlude the lumen of the afferent vessel. There is a marked increase in reticulum fibrils in contracted glomeruli.

The Capsular Basement Membrane: In the early stages it shows no change. Many authors have described a thickening where it turns

to be reflected over the glomerulus. This was not observed in any of the sixty cases. In later stages the reticular framework thickens and forms multiple concentric layers outside the inner apparently homogeneous basement membrane.

Capsular Epithelium: These cells react similarly to glomerular epithelium. In the early periods of the disease they become swollen, show mitoses and multiply rapidly. They often show hyaline granular degeneration. The intracellular fibrin clumps described by some authors seem on the contrary to be extracellular. The epithelium may form a very cellular single layer or multiple layers.

Crescent Formation: In the cases studied crescents were formed chiefly from proliferating capsular epithelium. When there is marked glomerular epithelial desquamation, the freed cells line the inner surface and may form the inner layers of the crescent. They may be distinguished from the capsular epithelium by their greater size, irregular shape, pedicles and dense cytoplasm. The regularly arranged outer portion of the crescent forms from capsular epithelium. Fibrin lies in threads, sheets and clumps between the cells. It appears bright red with the Mallory–Heidenhain stain. Blue hyaline fibers are also found in threads between the cells. These are very similar to the intracapillary fibers previously described. They have both knobs and knots at intersections. They soon form a meshwork in which the epithelium lies. This often occludes the convoluted tubule. Tubule-like cavities may appear in the crescent. The fibrin, polymorphonuclears and disintegrating desquamated glomerular epithelium soon disappear. The hyaline fibers seem to fuse with the capsular basement membrane. When adhesions develop between crescent and glomerulus, the hyaline fibers of the crescent are continuous with the glomerular basement membrane and through it with the intracapillary fibers. The closely related mesenchymal origin of all the cells in a glomerulus makes it theoretically possible for any one cell to produce fibrils. The origin of the fibers in the crescent is not yet determined. In many areas they seem to come from the capsular basement membrane, while in other places they appear to be forming intercellularly without attachment.

Hyalinization of the Glomerulus: The intracapillary hyaline fibers increase in number and caliber until they form the bulk of the intracapillary glomerular masses. The enclosed mononuclear cells are not allowed much space for cytoplasm. Sometimes the whole loop

is filled with the mass of fibers and cells all the way out to its basement membrane. This allows no lumen for the circulation. When all the loops in a glomerulus are as extensively involved, the nuclei are gradually squeezed out of existence; then adjacent loops fuse. At the same time, if crescents are present the hyaline fibers there increase and press on the enclosed cells until they too disappear. The capsular basement membrane, crescent, glomerular basement membrane and loop contents all fuse. There is a general contraction, and more nuclei disappear. Finally only a hyaline fibrous mass with very few nuclei remains. Even in this end stage it is usually possible with the Mallory–Heidenhain stain to differentiate the capsular and crescent fibers from those of glomerular origin.

Summary

From a microscopic study of glomeruli of sixty cases of acute and chronic glomerulonephritis the conclusions are as follows:

1. The intracapillary cellular increase is chiefly endothelial and is explained by the numerous mitoses. The leucocytes are polymorphonuclear and are much less numerous than the endothelial cells. A small amount of fibrin and débris is found in most of the capillaries.

2. The extracapillary changes consist of proliferation, degeneration and desquamation of glomerular and capsular epithelium. These are easily distinguished from intracapillary lesions because the glomerular basement membrane lies between.

3. Crescent formation may be due to capsular epithelial proliferation or to a combination of capsular proliferation and glomerular epithelial desquamation. In a very early period hyaline fibers appear between the cells and are soon continuous with the capsular basement membrane.

4. Hyaline fibers also appear inside the glomerular capillaries. They stain blue with the Mallory–Heidenhain azan-carmine, or with Ohmori's rein blau and not at all with silver. When first formed, they lie between the proliferating endothelial cells and are usually attached to the basement membrane. They appear as fine sharp lines with knobs at intersections. They soon form a definite meshwork enclosing the intracapillary cells. This fibrous meshwork is be-

lieved to be an important and typical finding in glomerulonephritis. It is the initial stage of the hyalinization of the glomerulus. The process takes place in all the glomeruli but not always to the same degree. When the loops are only partially occluded, blood continues to circulate along one side and the glomerulus has some function. When every loop is occluded, the circulation cannot continue. The fibers within the loop increase in number and caliber, many fuse, and the enclosed cells are atrophied by pressure. The glomerular loop arrives at a stage where it consists of a few flattened epithelial cells, a basement membrane and a lumen completely filled with a hyaline fibrous mass enclosing a few nuclei. Adjacent loops fuse, and the fibers contract. The glomerular epithelium atrophies and disappears. At the same time the fibers of the crescent have increased and contracted until they have eliminated most of the cells. The fibrous crescent now fuses with the fibrous glomerulus. The end result is a sphere of fibrous connective tissue containing practically no nuclei.

5. The origin and composition of the intracapillary hyaline fibers are undetermined and require further study. They do not seem to be fibrin or derivatives of it. They are thought to be a crystallization or precipitation of the intercellular exudate under the influence of the proliferating endothelial cells or perhaps a product of the glomerular basement membrane. Later they are indistinguishable from typical collagenous fibers.

6. The above processes are typical of clinical glomerulonephritis. The finding of a glomerular cellular increase (usually monocytes) following pneumonia and other infections is not to be confused with clinical nephritis. The non-clinical cases do not have the characteristic intracapillary fibrous meshwork.

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BIBLIOGRAPHY

Allen, R. Bernard. Reticular fibers in renal glomeruli. *Anat. Rec.*, Am. A. Anat. Abstr., 1927, xxv, 30.

Aschoff, L. Pathologische Anatomie. Ed. 5, 1921, part ii, 480.

Aufrecht. Die Bedeutung der nieren Glomeruli für die klinische Beurtheilung der primären Nierenentzundungen. *Berl. klin. Wchnschr.*, 1886, i, 7.

Aufrecht. Zum Nachweis zweier Nephritisarten. *Deutsches Arch. f. klin. Med.*, 1894, liii, 531–561.

Baitsell, G. A. The origin and structure of a fibrous tissue which appears in living cultures of adult frog tissues. *J. Exper. Med.*, 1915, xxi, 455–479.

Baitsell, G. A. The origin and structure of a fibrous tissue formed in wound healing. *J. Exper. Med.*, 1916, xxiii, 739–756.

Baitsell, G. A. A study of the clotting of the plasma of frog's blood and the transformation of the clot into a fibrous tissue. *Am. J. Physiol.*, 1917, xliv, 109–131.

Baitsell, G. A. A study of the development of connective tissue in the amphibia. *Am. J. Anat.*, 1921, xxviii, 447–466.

Baitsell, G. A., and Mason, Karl E. The origin of the fibrous tissue arising in the guinea pig testis as a result of experimental tuberculosis. *Tr. 22nd Annual Meeting of the National Tuberculosis A.*, 1926, 232–236.

Baitsell, G. A. Additional evidence as to intercellular formation of connective tissue. *Proc. Nat. Acad. Sc.*, 1927, xiii, 481–484.

Bargmann, W. Zur Morphologie des Nierenglomerulus. *Ztschr. f. Zellforsch. u. mikr. Anat.*, 1929, viii, 765–771.

Bell, E. T., and Hartzell, T. B. The etiology and development of glomerulonephritis. *Arch. Int. Med.*, 1922, xxix, 768–820.

Bidder, F. Über die Malpighischen Körper der Niere. *Arch. f. Anat. u. Physiol.*, 1845, 508–517.

Böhm, Arthur. Beitrag zur pathologischen Anatomie der Malpighi'schen Körperchen der Niere. *Virchows Arch. f. path. Anat.*, 1897, cl, 52–72.

Bowman, W. On the structure and use of the malpighian bodies of the kidney with observations on the circulation through that gland. *Phil. Tr.*, 1842, i, 57.

Bright, Richard. Reports of Medical Cases. London, 1827, i, 1–74.

Buday, K. Experimentell-histologische Studien über die Genese des Nierentuberkels. *Virchows Arch. f. path. Anat.*, 1906, clxxxvi, 145–212.

von Buhl, Ludwig. Epidemische Cholera. *Ztschr. f. Rationelle Med.*, 1855, vi, 1–105.

von Buhl, Ludwig. Über Bright's Granularschwund der Nieren und die damit zussammenhängende Herzhypertrophie. *Patholog. Institut zu München Mittheilungen*, Stuttgart, 1878, 38–91.

Carus, J. Victor. Über die Malpighischer Körper der Niere. *Ztschr. f. wissensch. Zool.*, 1850, ii, 58–61.

Christison, Robert. On Granular Degeneration of the Kidneys. Edinburgh, 1839.

Chrzonszczewsky, N. Zur Anatomie der Niere. *Virchows Arch. f. path. Anat.*, 1864, xxxi, 153–199.

Corner, G. W. On the widespread occurrence of reticular fibrils produced by capillary endothelium. *Contrib. Embryol.*, 1920, ix, 87–94.

Cornil, V. Nouvelles observations histologiques sur l'état des cellules du rein dans l'albuminurie. *J. de l'anat. et de la physiol.*, 1879, xv, 402–448.

Cornil, V., and Brault, A. De l'inflammation des glomérules dans les néphrites albumineuses. *J. de l'anat. et de la physiol.*, 1883, xix, 205–240.

Councilman, W. T. Forty-nine Cases of Acute and Subacute Nephritis. Boston, 1897.

Davidoff, L. M. Staining fibrillary neuroglia in formalin-fixed material. *Am. J. Path.*, 1928, iv, 493.

Dietrich, A. Überraschende Todesfälle durch Nephritis. *Klin. Wchnschr.*, 1917, liv, 521–523.

Drasch, Otto. Über das Vorkommen zweierlei verschiedener Gefässknäuel in der Niere. *Sitzungsb. d. kaiser. Akad. d. Wissenschaft. Math.-naturw. Cl.*, 1877, lxxvi, 79–102.

Duncan, Joh. Über die Malpighi'schen Knäuel in der Froschniere. *Sitzungsb. d. Wien. Akad.*, 1867, lvi, 7.

Dunin, Theodor. Anatomische Untersuchungen über Nierenentzündung und Morbus Brightii. *Virchows Arch. f. path. Anat.*, 1883, xciii, 286–342.

von Ebner, Victor. A. Koelliker's Handbuch der Gewebelehre des Menschen. 1902, iii, 363–378.

Eliaschoff, Ida. Über die Wirkung des Cantharidins auf die Nieren. *Virchows Arch. f. path. Anat.*, 1883, xciv, 323–342.

Elwyn, Herman. Nephritis. 1926.

Engel, H. Glomerulitis adhaesiva. *Virchows Arch. f. path. Anat.*, 1901, clxiii, 209–226.

Ernst, Paul. Über das Vorkommen von Fibrin in Nieren Cylindern. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1893, xii, 553–565.

Fahr, Th. Über herdförmige Glomerulonephritis. *Virchows Arch. f. path. Anat.*, 1918, ccxxv, 24–44.

Fahr, Th. Zur Frage der sogenannten hyalintropfigen Zelldegeneration. *Verhandl. d. deutsch. path. Gesellsch.*, 1914, xvii, 119–122.

Fahr, Th. Handbuch der speziellen pathologischen Anatomie und Histologie. Henke und Lubarsch, 1925, vi, 290–348.

Ferguson, J. B. The application of the silver impregnation method of Bielschowsky to reticular and other connective tissues. *Am. J. Anat.*, 1911, xii, 277–296.

Foot, Nathan Chandler. Studies on endothelial reactions. V. The endothelium in the healing of aseptic wounds in the omentum of rabbits. *J. Exper. Med.*, 1921, xxxiv, 625–641.

Foot, Nathan Chandler. Studies on endothelial reactions. IX. The formation of reticulum in the lesions of experimental tuberculosis in rabbits. *Am. J. Path.*, 1925, i, 341–349.

Foot, Nathan Chandler. The occurrence of reticulum in tumors. *Am. J. Path.*, 1925, i, 431–443.

Foot, Nathan Chandler. On the origin of reticulum fibrils. *Am. J. Path.*, 1927, iii, 401–411.

Frerichs, Th. Die Bright'sche Nierenkrankheit und deren Behandlung. Braunschweig, 1851, 180–276.

Frey, Heinrich. The Histology and Histochemistry of Man. Translated from the fourth German edition by Arthur E. Barker, N. Y., 1875, 516–530.

Friedländer, Carl. Über Nephritis scarlatinosa. *Fortschr. d. Med.*, 1883, i, 81–94.

von Frisch, Bruno. Zum feineren Bau der Membrana propria der Harnkanälchen. *Anat. Anz.*, 1915, xlviii, 284–296.

Gaskell, J. F. On the changes in glomeruli and arteries in inflammatory and arteriosclerotic kidney disease. *J. Path. & Bact.*, 1911–12, xvi, 287–320.

Gerlach, J. Beiträge zur Strukturlehre der Niere. *Arch. f. Anat. u. Physiol. u. wissensch. Med.*, 1845, 377–386.

Gerlach, J. *Arch. f. Anat. u. Physiol. u. wissensch. Med.*, 1848, 102–112.

Goemans, J. L. La capsule de Bowman dans les inflammations chroniques des reins. *Rec. de trav. anat.-path. du lab. Boerhaave*, 1899, ii, 424–471. Quoted by Engel, 1901.

Gräff, Siegfried. Untersuchungen über das Verhalten der Leukozyten im Glomerulusgebiet bei der akuten Glomerulonephritis. *Deutsche med. Wchnschr.*, 1916, xlii, 1092–1093.

Greenfield, W. S. Granular contracted kidney. *Tr. Path. Soc. London*, 1880, xxxi, 157–164.

Gross, W. Frische Glomerulonephritis. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1919, lxv, 387–422.

Hansemann, D. Zur pathologischen Anatomie der Malpighi'sche Körperchen der Niere. *Virchows Arch. f. path. Anat.*, 1887, cx, 52–80.

Hedinger, H. Über den Bau der Malpighi'schen Gefässknäuel der Niere. Inaugural Dissertation, Breslau, 1888.

Heidenhain, M. Über die Mallorysche Bindegewebsfärbung mit Karmin und Azokarmin als Vorfarben. *Ztschr. f. wissensch. Mikr.*, 1915, xxxii, 361–372.

Heidenhain, R. Mikroskopische Beiträge zur Anatomie und Physiologie der Nieren. *Arch. f. Mikr. Anat.*, 1874, x, 1–49.

Henle, J. Zur Anatomie der Niere. Göttingen, 1862.

Henle, J. Handbuch der systematischen Anatomie des Menschen. 1873, ii, 300–333.

Herxheimer, G. Über die sogenannte hyaline degeneration der Glomeruli der Niere. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1909, xlv, 253–282.

Herxheimer, G. Über das pathologisch-anatomische Bild der Kriegsnephritis. *Deutsche med. Wchnschr.*, 1916, xlii, 869-870, 906–908, 940–942, 969–970.

Herxheimer, G. Über den jetzigen Stand unserer anatomischen Kenntnisse der Nephritis und Nephropathien. *München. med. Wchnschr.*, 1918, xi, 283.

Herxheimer. G. Nierenstudien ii. Über Anfangsstudien der Glomerulonephritis. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1918, lxiv, 454–476.

Herring, Percy D. The development of the malpighian bodies of the kidney and its relation to pathological changes which occur in them. *J. Path. & Bact.*, 1900, vi, 459–496.

His, W. Die Häute und Höhlen des Korpers. Basel, 1865.

Hlava, Jar., and Thomayer, Jos. Über die pathologisch-anatomischen Bedingungen des Urämischen Symptomen-Complexes bei Nephritiden. *Med. Jahr.*, 1882, 213.

Hortoles, Ch. Recherches histologiques sur le glomérule et les épithéliums du rein. *Arch. de Physiol.*, 1881, ii, ser. xiii Année, 861–885.

Huber, G. C. The morphology and structure of the mammalian renal tubule. The Harvey Lectures, 1909–1910, 110–149.

Hückel, R. Die Veränderungen im Beginn der diffuse Glomerulonephritis. *München med. Wchnschr.*, 1928, xxxv, 1526.

Hueter, C. Über amyloid der Glomeruli. *Centralbl. f. allg. Pathol. u. path. Anat.*, 1908, xix, 961–965.

Hung, See Lü. Über eine Methode zur Darstellung der Basalmembranen. *Virchows Arch. f. path. Anat.*, 1922, ccxl, 355–360.

Huschke, E. Über die Textur der Niere. Isis, 1828. Quoted by Henle, 1873.

Isaacs, Charles E. Researches into the structure and physiology of the kidney. *Tr. New York Acad. Med.*, 1857, i, 377–435.

Johnson, G. On the inflammatory diseases of the kidney. *Medico-Chir. Tr.*, 1847, xxx, 160–189.

Johnson, G. On the proximate cause of albuminous urine and dropsy. *Medico-Chir. Tr.*, 1850, xxxiii, 107–120.

Johnson, G. Lecture on the pathology, diagnosis and treatment of Bright's disease. *Brit. M. J.*, 1873, i, 81–82, 275–277.

Johnston, W. B. A reconstruction of a glomerulus of the human kidney. *Anat. Anz.*, 1899, xvi, 260–266.

Jungmann, Paul. Über akute Nierenerkrankungen bei Kreigsteilnehmern. *Ztschr. f. klin. Med.*, 1917, lxxxiv, 1–21.

von Kahlden, C. Die Aetiologie und Genese der acuten Nephritis. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1891, xi, 441–592.

von Kahlden, C. Über die Glomerulonephritis bei Scharlach. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1894, xv, 602–625.

Kaufmann, Eduard. Lehrbuch der speciellen pathologischen Anatomie. 1911, ii, 830.

Kelsch, A. Revue critique et recherches anatomo-pathologiques sur la maladie de Bright. *Arch. de Physiol.*, 1874, 722–758.

Key, E. Axel. Om Circulationsförhållandena i Njurarne. *Förh. v. de. skandin. Naturf.*, Stockholm, 1865, 685–725. Quoted by Ludwig, 1872.

Kiener and Kelsch. Les alterations paludesmes du rein. *Arch. de physiol.*, Series ii, 1882, ix, 278–323, 458–498.

Klebs, Edwin. Handbuch der pathologischen Anatomie. 1863, 644.

Klebs, Edwin. Die allgemeine Pathologie. 1889, ii, 415–419.

Klein, E. The anatomical changes of the kidney, liver, spleen and lymphatic glands in scarlatina of man. *Tr. Path. Soc. London*, 1877, xxviii, 430–440.

von Koelliker, A. Mikroskopische Anatomie oder Gewebelehre des Menschen. 1854, vol. ii.

Krauspe, Carl. Beiträge sur Kenntnis der Gitterfasern mit besonderer Berücksichtigung der Niere. *Virchows Arch. f. path. Anat.*, 1922, ccxxxvii, 475–491.

Kuczynski, Max H., and Dosquet, H. Zweiter anatomischer Beitrag zur Pathogenese der Glomerulonephritis. *Krankheitsforschung*, 1926, iii, 201–248.

Langhans, Th. Über die Veränderungen der Glomeruli bei Nephritis nebst einigen Bemerkungen über die Entstehungen der fibrin Cylinder. *Virchows Arch. f. path. Anat.*, 1879, lxxvi, 85–118.

Langhans, Th. Über die entzundlichen Veränderungen die Glomeruli und die acute Nephritis. *Virchows Arch. f. path. Anat.*, 1885, xcix, 193–250.

Lee-Brown, R. K., and Laidly, J. W. S. Some observations on the microscopical anatomy of the kidney. *J. Urol.*, 1929, xxi, 259–274.

Leyden, E. Über das erste Stadium des Morbus Brightii und die akute oder frische Nephritis. *Ztschr. f. klin. Med.*, 1881, iii, 161–208.

Litten, M. Über Scharlachnephritis. *Charité-Ann.*, 1878, iv, 179–188.

Littre, Alexis. Observation sur les reins d'un foetus humain de neuf mois. *Mém. Acad. roy. d. sc. de Par.*, 1705. Quoted by Bowman, 1842.

Löhlein, M. Über Nephritis nach dem heutigen Stande der pathologisch-anatomischen Forschung. *Ergebn. d. inn. Med. u. Kinderh.*, 1910, v, 411–458.

Ludwig, Carl. Lehrbuch der Physiologie des Menschen. 1861, ii, 373.

Ludwig, Carl. Manual of Human and Comparative Histology. S. Stricker, The New Sydenham Society, London, 1872, ii, 83–106.

Luna. Studio sul tessuto reticolare. *Ricerche di morfol.*, 1920, i, 191. Quoted by Bargmann, 1929.

MacCallum, W. G. A Textbook of Pathology. 1916.

McClung, C. E. Handbook of Microscopical Technique. 1929.

McDonnell, R. Observations on the Anatomy and Physiology of the kidney. *Glasgow M. J.*, 1855, ii, 285–295.

Mall, Franklin P. Reticulated tissue and its relation to the connective tissue fibrils. *Rep. Johns Hopkins Hosp.*, 1896, i, 171–208.

Mall, Franklin P. On the development of the connective tissues from the connective tissue syncytium. *Am. J. Anat.*, 1902, i, 329–365.

Mallory, F. B. The Principles of Pathologic Histology. Philadelphia, 1914.

Mallory, F. B., and Parker, F. Reticulum. *Am. J. Path.*, 1927, iii, 515.

Malpighi, M. De renibus Exercit de visc. struct., 1688. Quoted by Bowman, 1842.

Maresch, Rudolf. Über Gitterfasern der Leber und die Verwendbarkeit der Methode Bielschofskys zur Darstellung feinster Bindegewebsfibrillen. *Centralbl. f. allg. Pathol. u. path. Anat.*, 1905, xvi, 641–649.

Maximow, Alexander. Über die Entstehung von argyrophilen und kollagenen Fasern in Kulturen von Bindegewebe und von Blutleukozyten. *Centralbl. f. allg. Pathol. u. path. Anat.*, 1928, xliii, 145–152.

Merkel, F. Betrachtungen über die Entwickelung des Bindegewebes. *Anat. Hefte*, 1909, xxxviii, 323–383.

Mertz, Albrecht. Über die quantitative Zellverhältnisse der Glomeruli bei Glomerulonephritis. *Centralbl. f. allg. Pathol. u. path. Anat.*, 1918, xxix, 321–341.

Miller, Wm. Snow. Studies on the normal and pathological histology of the lung. The Harvey Lectures, 1924, 42–59.

Miller, Wm. Snow. The reticulum of the lung. IV. *Am. J. Path.*, 1927, iii, 217–224.

Miller, Wm. Snow. The reticulum of the lung. V. *Am. J. Path.*, 1927, iii, 315–319.

Moleschott, Jac. Ein histochemischer und ein histologischer Beitrag zur Kenntnis der Nieren. *Untersuch. z. Naturl. d. Mensch. u. d. Thiere*, 1862, viii, 213–224.

von Moellendorff, Wilhelm. Einige Beobachtungen über den Aufbau des Nierenglomerulus. *Ztschr. f. Zellforsch. u. mikr. Anat.*, 1927, vi, 441–450.

Müller, F. Morbus Brightii. *Verhandl. d. deutsch. path. Gesellsch.*, 1905, ix, 64–99.

Müller, Joh. De glandularum secernentium structura penitiori. Leipzig, 1830.

Munk, Fritz. Nieren Erkrankungen. Berlin, 1925.

Nauwerck, C. Über Endothelveränderungen bei acuter Nephritis. *Deutsche med. Wchnschr.*, 1884, x, 166–188.

Nauwerck, C. Beiträge zur Kenntnis des Morbus Brightii. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1886, i, 5–84.

Nussbaum, M. Über den Bau und die Thätigkeit der Drusen. *Arch. f. mikr. Anat.*, 1886, xxvii, 442–460.

Obrzut, Andre. Nouvelles recherches sur la pathogenie de la glomerulonephrite. *Rev. de méd.*, 1888, viii, 689–712.

Ohmori, D. Bedeutung der Membrana Propria der Glomerulus Schlingen in der Nierenpathologie. *Virchows Arch. f. path. Anat.*, 1921, ccxxxiv, 53–71.

Orsós, F. Das Bindegewebsgerüst der Lymphoknoten im normalen und pathologischen Zustand. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1926, lxxv, 15–134.

Orsós, F. Das Bindegewebsgerüst des Knochenmarks im normalen und pathologischen Zustand. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1926, lxxvi, 36–86.

Orth, Johannes. Lehrbuch der speziellen pathologischen Anatomie. Berlin, 1893, ii, 45–94.

Perdrau, J. R. The silver reduction method for the demonstration of connective tissue fibers. *J. Path. & Bact.*, 1921, xxiv, 117.

Posner, Carl. Studien über pathologische Exsudatbildungen. *Virchows Arch. f. path. Anat.*, 1880, lxxix, 311–382.

Randerath, Edmund. Über die Beteiligung der Glomeruluskapsel bei der diffusen Glomerulonephritis. *Virchows Arch. f. path. Anat.*, 1929, cclxxi, 197–210.

Rayer, P. F. O. Traité des maladies des reins, Paris, 1837–41. Quoted by Orth, 1893.

Regaud, Cl., and Policard, A. Recherches sur la structure du rein de quelques ophidiens. *Arch. d'anat. micr.*, 1903–04, vi, 190–282.

Reichel, Heinrich. Über Nephritis bei Scharlach. *Ztschr. f. Heilk.*, 1905, xxvi, 72–102.

Reichert, K. B. Bericht über die Fortschritte der mikroskopische Anatomie in dem Jahre 1842. *Arch. f. Anat. Physiol. u. wissensch. Med.*, 1843, 180–238.

Ribbert, Hugo. Über die Entwickelung der Glomeruli. *Arch. f. mikr. Anat.*, 1879, xvii, 113–124.

Ribbert, Hugo. Beiträge zur pathologischen Anatomie der Glomeruli. *Fortschr. d. Med.*, 1888, vi, 490–500.

Rochs, K. Ein Beitrag zur Kenntnis der hëmorrhagischen Glomerulonephritis. *Virchows Arch. f. path. Anat.*, 1918, ccxxv, 60–88.

Rokitansky, Carl. A Manual of Pathological Anatomy. Sydenham Society, London, 1849, ii, 185–210.

Roth, M. Untersuchungen über die Drusensubstanz der Niere. *Schweiz. Ztschr. f. Heilk.*, 1864, iii, 1–33.

Roth, Ernst. Über Schrumpfinieren ohne Arteriosklerose. *Virchows Arch. f. path. Anat.*, 1907, clxxxviii, 527–550.

Rühle, Georg. Über die Membrane Propria der Harncanälchen und ihre Beziehung zu dem interstitiellen Gewebe der Niere. *Arch. f. Anat. u. Entwcklngsgesch.*, 1897, 153–170.

Russakoff, A. Über die Gitterfasern der Lunge unter normalen und pathologischen Verhaltnissen. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1908, xlv, 476–505.

Saundry, Robert. The histology of the granular kidney. *Tr. Path. Soc. London*, 1880, xxxi, 148–157.

Schäfer, E. A. Quain's Elements of Anatomy. 1912, ii, 590–597.

Schäffer, J. Das Epithelgewebe. Handbuch der mikroskopische Anatomie des Menschen. 1927, i, 72–75.

Schumlansky, Al. Anatomica de renum structura. Argentorati. Inaugural Dissertation, 1782. Quoted by Bowman, 1842.

Schweigger-Seidel, F. Die Niere des Menschen und der Saugethiere in ihrem feinerem Bau. Halle, 1865. Quoted by von Ebner, 1902.

Seng, Victor. Ein Beitrag zur Lehre von dem Malpighi'schen Körperchen der menschlichen Niere. *Sitzungsb. d. Kaiser. Akad. d. Wissenschaft.*, 1871, lxiv, 354–358.

Sorenson, S. T. Über scharlach Nephritis. *Ztschr. f. klin. Med.*, 1891, xviii, 298–343.

Steudener, Frid. Nonnulla de penitiore renum structura et physiologica et pathologica. Inaugural Dissertation, Halle, 1864.

Thoma, Richard. Zur Kenntniss der Circulationsstörung bei chronischer interstitieller Nephritis. *Virchows Arch. f. path. Anat.*, 1877, lxxi, 227–248.

Toldt, C. Untersuchungen über das Wachsthum der Nieren des Menschen und der Säugethiere. *Sitzungsb. d. Kaiser. Akad. d. Wissenschaft.*, 1874, lxix, 123–148.

Toynbee, J. On the intimate structure of the human kidney and on the changes which its several component parts undergo in Bright's disease. *Medico-Chir. Tr.*, 1846, xxix, 303–326.

Tschistowitsch, Th. Die Verodung und hyaline Entartung der Malpighischen Körperchen der Niere. *Virchows Arch. f. path. Anat.*, 1903, clxxi, 243–257.

Vimtrup, B. J. On the number, shape, structure and surface area of the glomeruli in the kidneys of man and mammals. *Am. J. Anat.*, 1928, xli, 123–147.

Virchow, Rudolph. Über parenchymatöse Entzündung. *Virchows Arch. f. path. Anat.*, 1852, iv, 261–324.

Volhard and Fahr. Die Brightsche Nierenkrankheit. Berlin, 1914.

Volterra, M. Über die Struktur des Nierenglomerulus. *Ztschr. f. Zellforsch. u. mikr. Anat.*, 1928, vii, 135–140.

Waller, Bryan Charles. On the morbid anatomy of certain forms of post scarlatinal nephritis. *J. Anat. & Physiol.*, 1880, xiv, 432–448.

Weigert, Carl. Die Bright'sche Nierenerkrankungen vom pathologisch-anatomischen Standpunkte. *Samml. klin. Vortr.*, 1879, lv, 1411–1459.

Welch, W. H. An experimental study of glomerulonephritis. *Tr. A. Am. Phys.*, 1886, i, 170–183.

White, C. E., and Smith, M. I. The relation of blood clotting to tubercle structures. *Tr. Am. Clin. & Climat. A.*, 1924, xl, 186–188.

Wilks, Samuel. Cases of Bright's Disease. *Guy's Hosp. Rep.*, 1853, vii, 232–315.

von Wittich. Beiträge zur Anatomie der gesunden und kranken Niere. *Virchows Arch. f. path. Anat.*, 1851, iii, 142–153.

Ziegler, E. Über die Ursachen der Nierenschrumpfung nebst Bemerkungen über die Unterschiedung verschiedener Formen der Nephritis. *Deutsches Arch. f. klin. Med.*, 1879, xxv, 586–623.

Zimmermann, K. W. Zur Morphologie der Epithelzellen der Säugetierniere. *Arch. f. mikr. Anat.*, 1911, lxxviii, 199–229.

Zimmermann, K. W. Über das epithel des glomerularen Endkammerblattes der Säugerniere. *Anat. Anz.*, 1915, xlviii, 335–341.

Zimmermann, K. W. Die feinere Bau der Blutcapillaren. *Ztschr. f. Anat. u. Entwcklngsgesch.*, 1923, lxviii, 30–109.

DESCRIPTION OF PLATES

PLATE 105

FIG. 1. One loop of a normal glomerulus. Gl. ep. = glomerular epithelium; gl. b. m. = glomerular basement membrane; end. = endothelium; r. b. c. = red blood cell; l. = leucocyte.

FIG. 2. One loop of a glomerulus from a case of acute glomerulonephritis. Note the swollen, glomerular epithelium, the large endothelial cells in several layers and the intracapillary hyaline fibers. C. b. m. = capsular basement membrane; c. ep. = capsular epithelium; gl. ep. = glomerular epithelium; gl. b. m. = glomerular basement membrane; end. = endothelium; end. 2. = proliferating endothelium; hy. f. = hyaline intracapillary fibers.

FIG. 3. One loop of a glomerulus from a later stage of acute glomerulonephritis. Gl. ep. = glomerular epithelium; gl. b. m. = glomerular basement membrane; end. = endothelium; end. 2 = proliferated endothelium; hy. f. = intracapillary hyaline fibers.

Rbc
L
End
Gl Ep
Gl BM
Fig. 1
CBM
C Ep
End 2
Hy F
Gl BM
Rbc
End
Gl Ep
Fig. 2
Gl BM
Gl Ep
End 2
End
Hy F
Fig. 3

Plate 106

Fig. 4. One loop from a glomerulus of chronic glomerulonephritis. Gl. ep. = glomerular epithelium; gl. b. m. = glomerular basement membrane; end. = endothelium; end. 2 = proliferated endothelium; r. b. c. = red blood cell; hy. f. = hyaline fibers.

Fig. 5. One loop from a glomerulus of chronic glomerulonephritis. C. b. m. = capsular basement membrane; gl. ep. = glomerular epithelium; end. = endothelium; end. 2 = proliferated endothelium; hy. f. = intracapillary hyaline fibers.

Figures 1, 2, 3, 4 and 5 are camera lucida drawings from oil immersion fields.

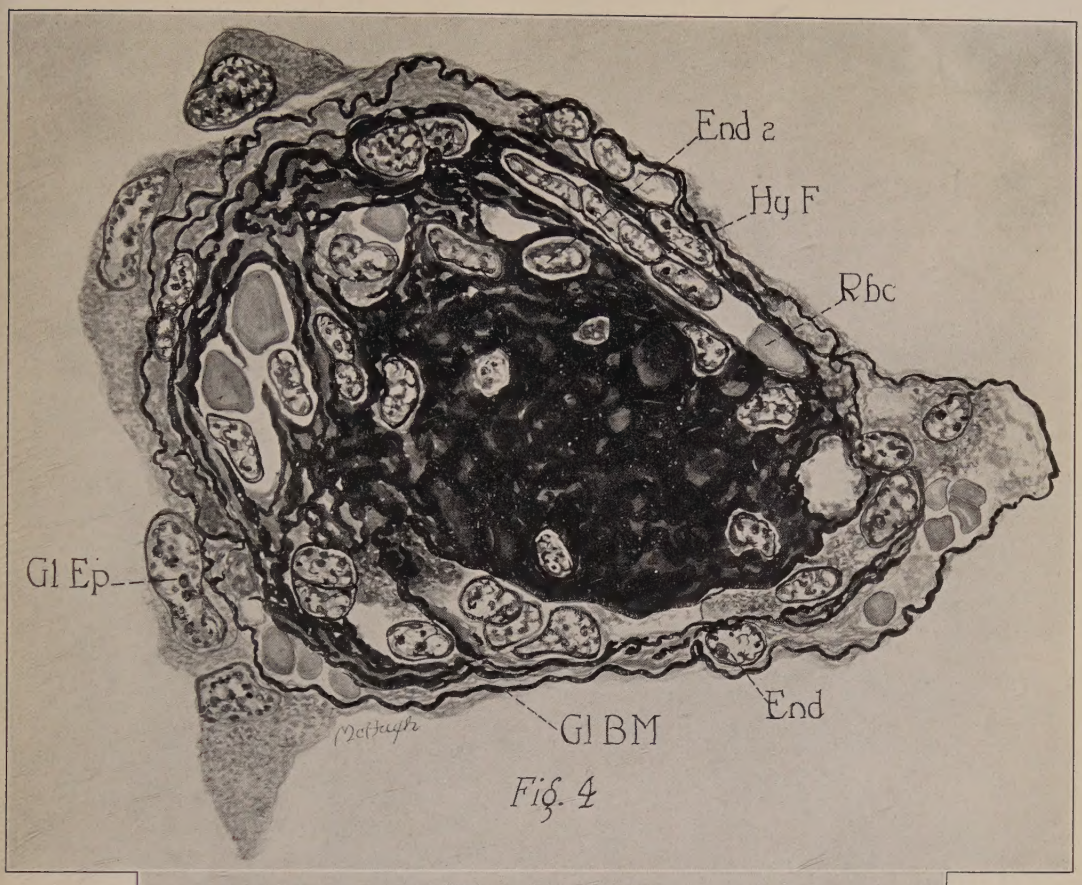

Fig. 4

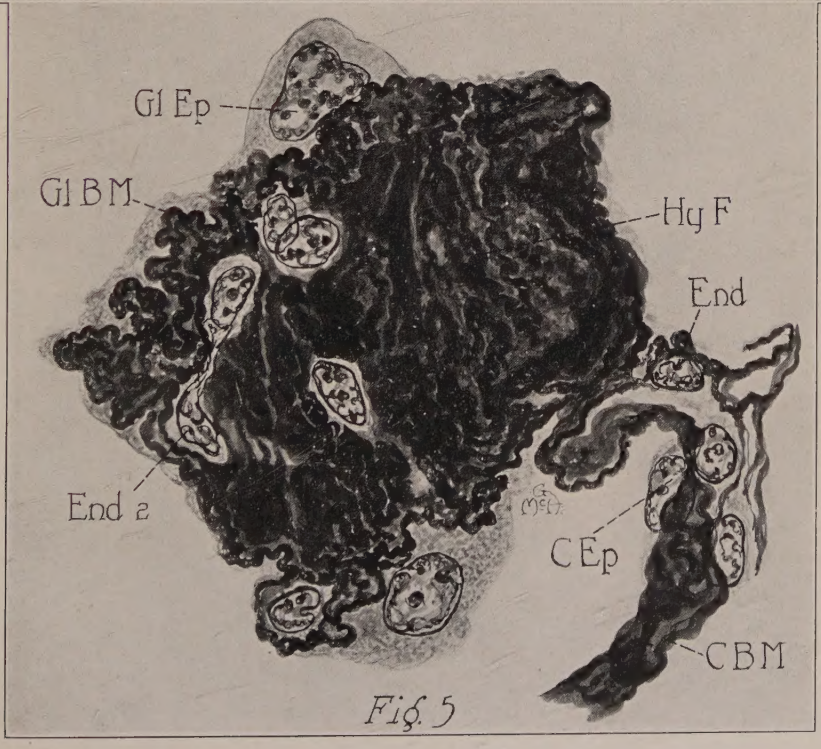

Fig. 5

VITA

The author, Leone McGregor, was born on January 19, 1900 in Oarnduff, Sask., Canada. She was graduated from the Calgary (Alberta, Canada) High School in 1918. She received the degree of M.D. in 1925 from the University of Alberta. She entered the Graduate School of the University of Minnesota in 1926, and received the degree of Master of Science in 1927, and the degree of Doctor of Philosophy in 1929. She was a teaching fellow in the Pathology Department, University of Minnesota from 1926 to 1929. She is now a Fellow on the National Research Council, Boston City Hospital, Boston, Mass.